ERAP1-ERAP2 dimers trim MHC I-bound precursor peptides; implications for understanding peptide editing

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Supplementary Figure 1, Related to Figure 2 N-terminal trimming of free and HLA-B*0801-bound (RA)₂ALRSRYWAI by ERAP1/2. (**a**) Free (RA)₂ALRSRYWAI 13mer was incubated with ERAP1/2 at 37°C. An aliquot was taken from the mixture after 1 hour 20 minutes. (**b**) HLA-B*0801-bound (RA)₂ALRSRYWAI 13mer was incubated with ERAP1/2 at 37°C. Aliquots were taken from the mixture after 2, 6, and 10 hours. Initially, the enzyme:substrate ratio was as identical as possible in (**a**) and (**b**), but additional ERAP1/2 was added in (**b**) after 2 and 6 hours. (**c**) HLA-B*0801-bound (RA)₂ALRSRYWAI 13mer was incubated alone for 10 hours as in (**b**), in the absence of ERAP1/2. (**d**) HLA-B*0801-bound (RA)₂ALRSRYWAI 13mer was incubated with an excess of free AAKKKYKL 8mer ($m/z = 949$) for 10 hours as in (**b**), in the absence of ERAP1/2. The starting precursor peptide and its fragments are identified by their m/z values.

Supplementary Figure 2, Related to Figure 2 N-terminal trimming of free and HLA-B*0801-bound (RA)₃AAKKKYKL by ERAP1/2. (**a**) Free (RA)₃AAKKKYKL 14mer was incubated with ERAP1/2 at 37°C. An aliquot was taken from the mixture after 1 hour 20 minutes. (**b**) HLA-B*0801-bound (RA)₃AAKKKYKL 14mer was incubated with ERAP1/2 at 37°C. Aliquots were taken from the mixture after 2, 6, and 10 hours. Initially, the enzyme:substrate ratio was as identical as possible in (**a**) and (**b**), but additional ERAP1/2 was added in (**b**) after 2 and 6 hours. The starting precursor peptide and its fragments are identified by their m/z values.

Supplementary Figure 3, Related to Figure 2 N-terminal trimming of free and HLA-B*0801-bound (RA)₂AAKKKYKL by ERAP1/2. (**a**) Free (RA)₂AAKKKYKL 12mer was incubated with ERAP1/2 beads at 37°C. An aliquot was taken from the mixture after 1 hour 20 minutes. (**b**) HLA-B*0801-bound (RA)₂AAKKKYKL 12mer was incubated with ERAP1/2 at 37°C. Aliquots were taken from the mixture after 2, 6, and 10 hours. Initially, the enzyme:substrate ratio was as identical as possible in (**a**) and (**b**), but additional ERAP1/2 were added in (**b**) after 2 and 6 hours. The starting precursor peptide and its fragments are identified by their *m/z* values.

Supplementary Figure 4, related to Methods N-terminal trimming of free and HLA-B*0801-bound (RA)₃ALRSRYWAI by ERAP1/2 carrying inactive ERAP1. (**a**) Free (RA)₃ALRSRYWAI 15mer was incubated with ERAP1/2 (inactive/active) at 37°C. An aliquot was taken from the mixture after 1 hour 20 minutes. (**b**) HLA-B*0801-bound (RA)₃ALRSRYWAI 15mer was incubated with ERAP1/2 (inactive/active) at 37°C. Aliquots were taken after 2 and 10 hours. Initially, the enzyme:substrate ratio was as identical as possible in (**a**) and (**b**), but additional ERAP1/2 (inactive/active) was added in (**b**) after 2 and 6 hours. (**c**) Free (RA)₃ALRSRYWAI 15mer was incubated with ERAP1 (without Jun) and ERAP1-Jun at 37°C. An aliquot was taken from the mixture after 1 hour 20 minutes. The starting precursor peptides and their fragments are identified by their *m/z* values.

Supplementary Figure 5, Related to Figure 4 N-terminal trimming of HLA-B*0801-bound LAKLRNKLVI 10mer and AKLRNKLVI 9mer by ERAP1/2. (**a**) HLA-

B*0801/LAKLRNKLVI was incubated with ERAP1/2 at 37°C. Aliquots were taken from the mixture after 2 and 4 hours. (**b**) Same experiment as in (**a**) for HLA-B*0801/AKLRNKLVI. The enzyme:substrate ratio was as identical as possible in (**a**) and (**b**), and additional ERAP1/2 was added in (**a**) and (**b**) after 2 and 4 hours. The starting precursor peptides and its fragments are identified by their *m/z* values.
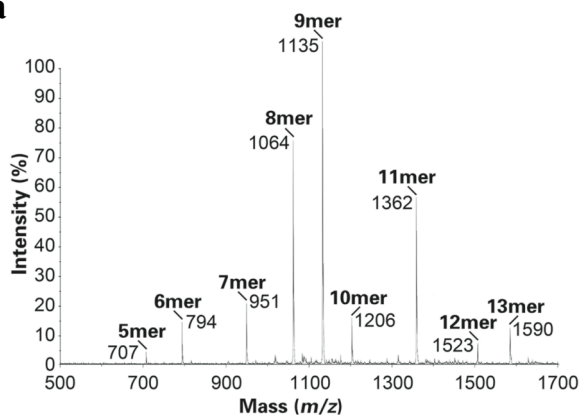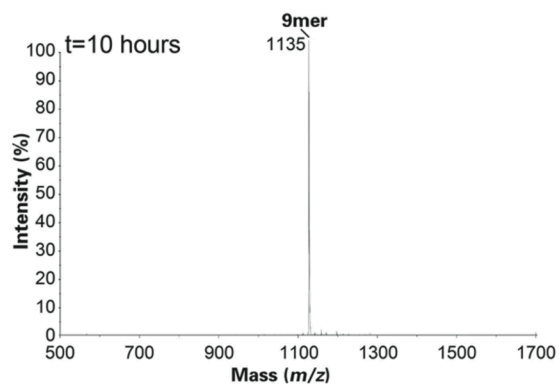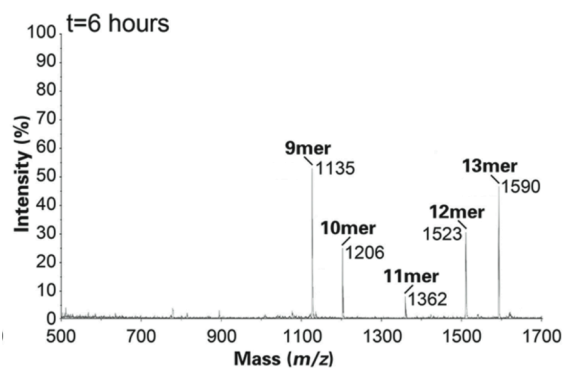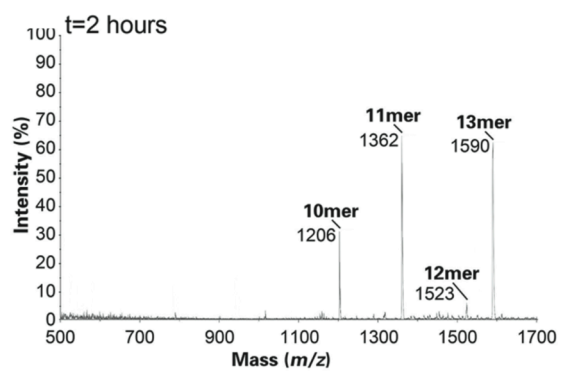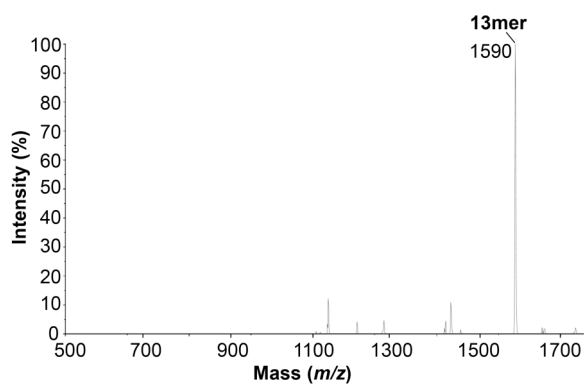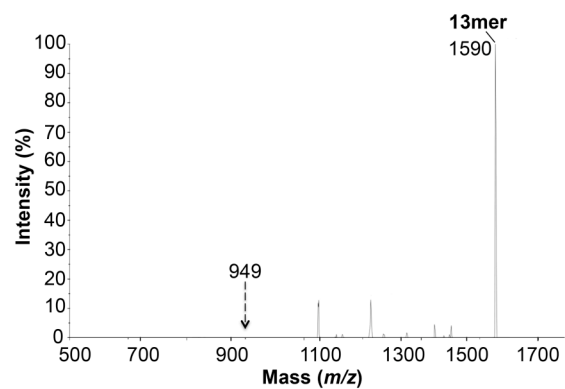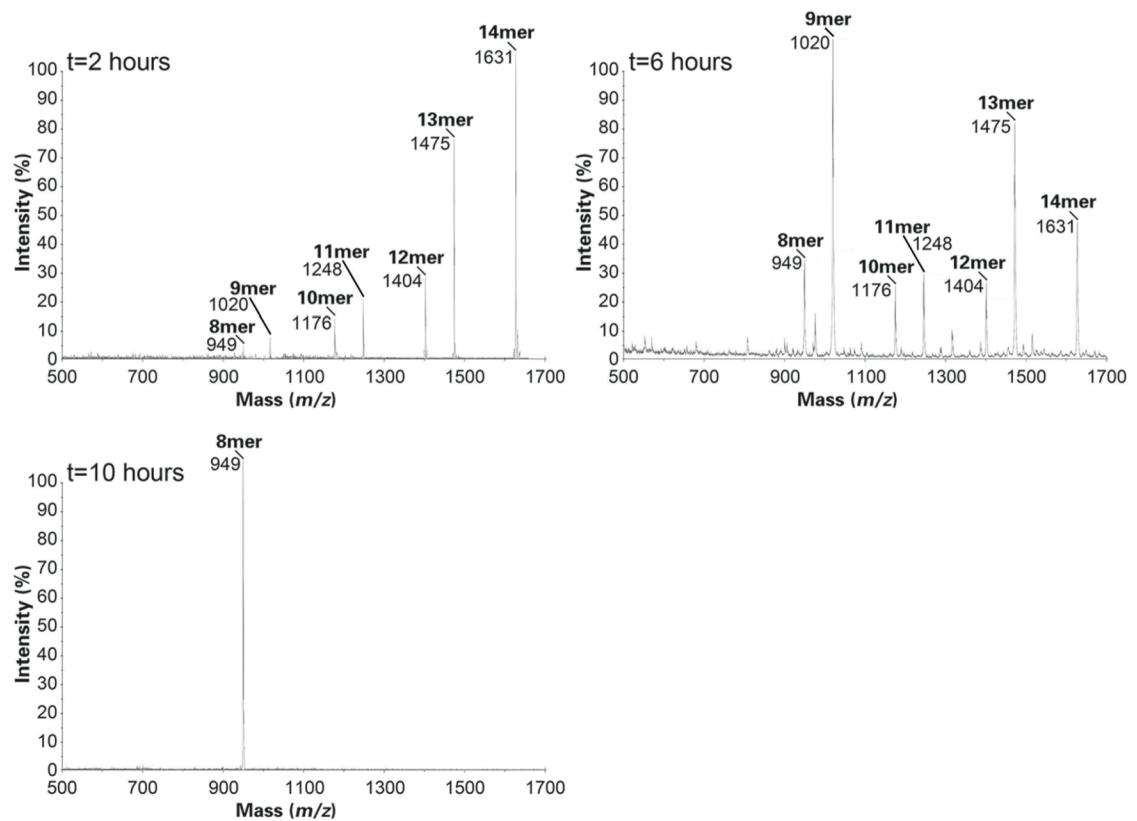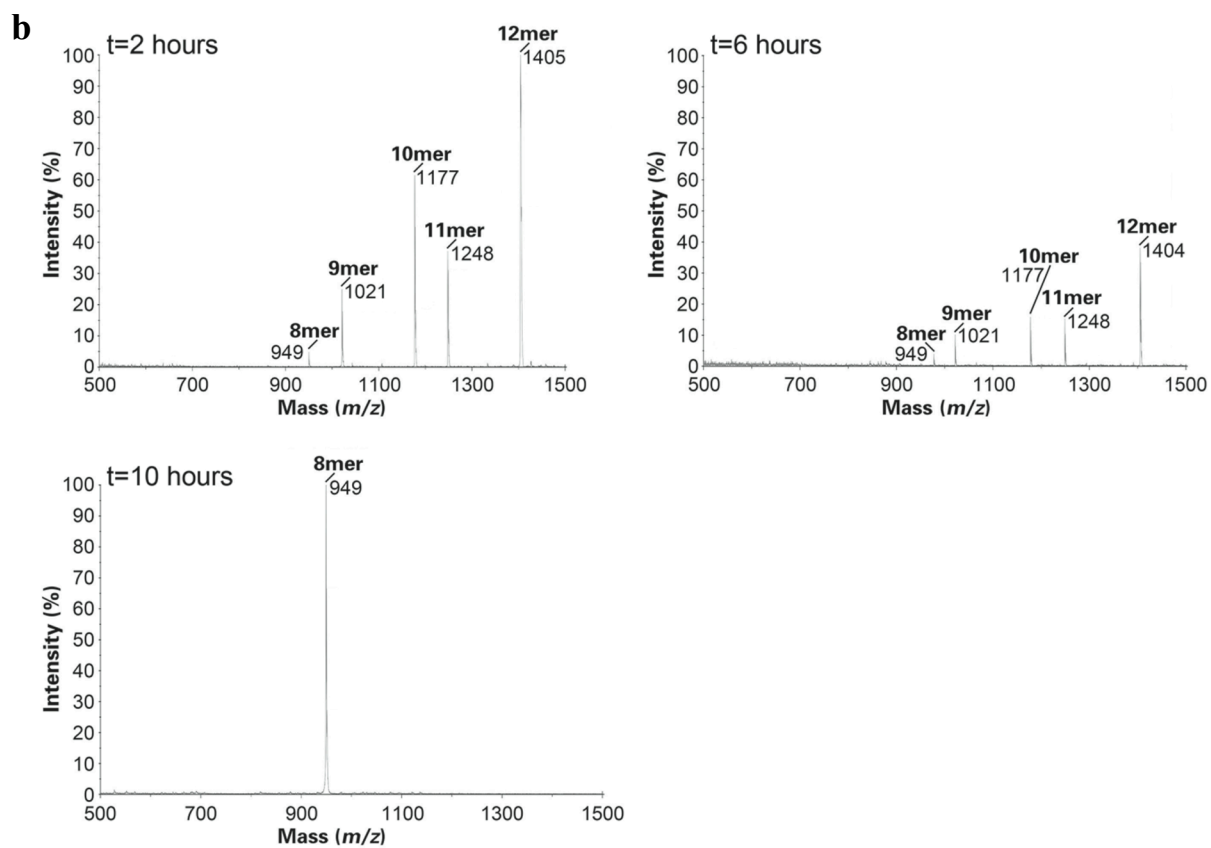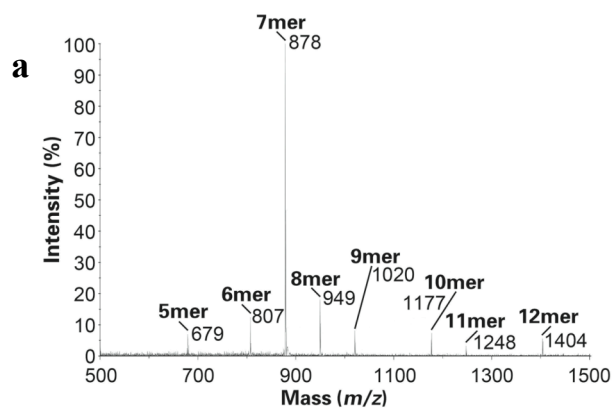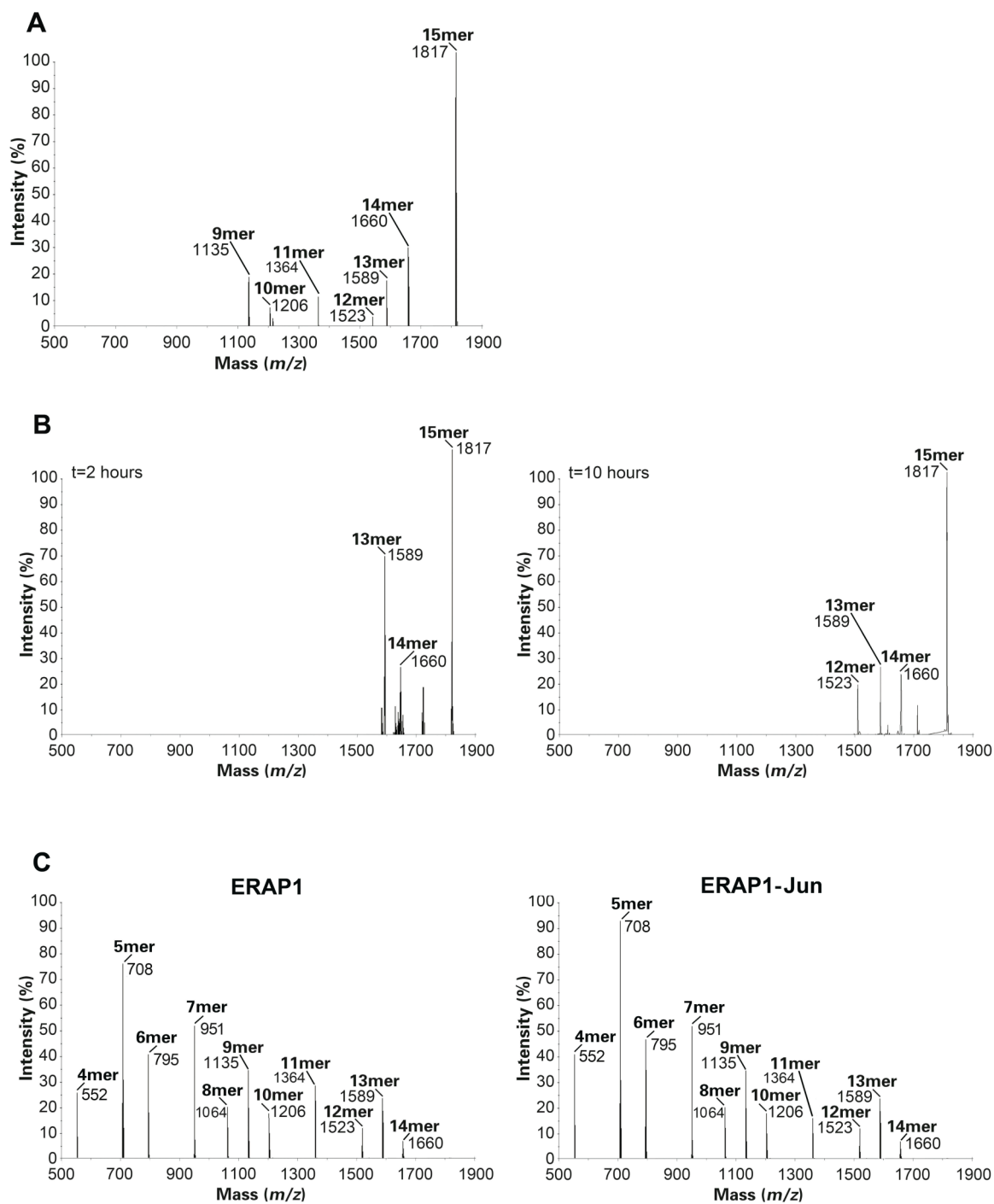
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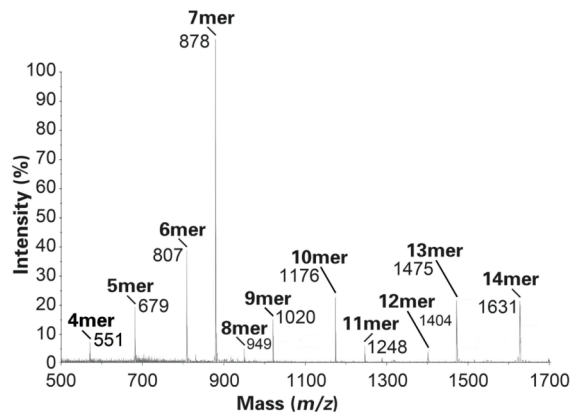
Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4
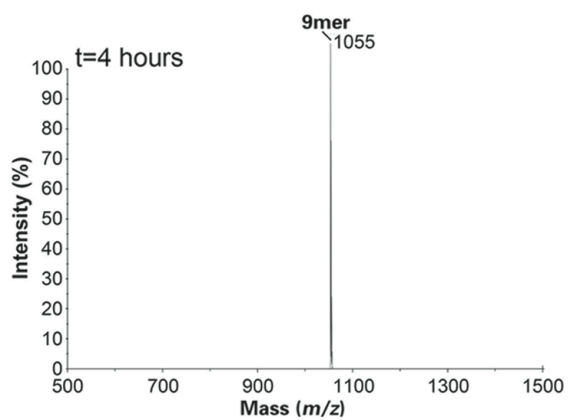
a

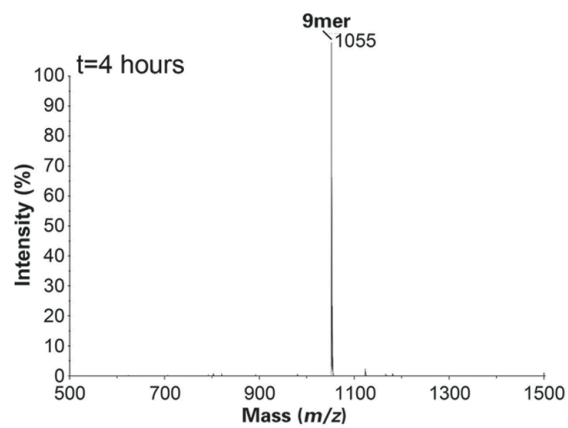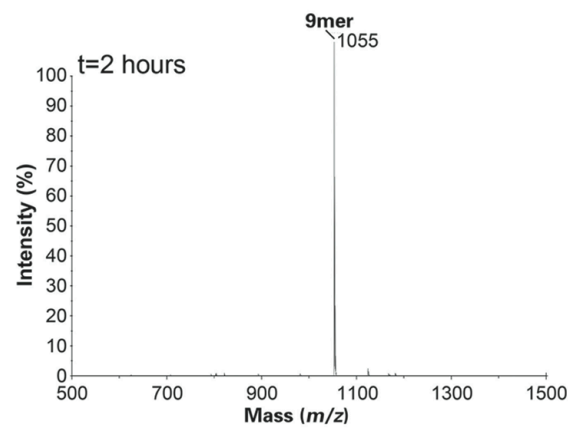HLA-B*0801/10mer

t=2 hours

9mer
1055

10mer
1168

t=4 hours

9mer
1055

b

HLA-B*0801/9mer

t=2 hours

9mer
1055

t=4 hours

9mer
1055

Supplementary Figure 5